Chapter 3 : Principles of Bacterial Taxonomy

Introduction

Taxonomy is the science of the laws of classification. It was in the 18th century that Linnaeus first proposed the principles of a so-called *Natural* classification for organisms in the animal and plant kingdoms. Then, in the 19th century, Ernst Haeckel classified protists as a third kingdom according to Linnaeus' principles. His principles state that every individual belongs to a species, every species to a genus, and so on, with the species being the foundation of the classification.

In microbiology, this allows us to identify microorganisms in order to better utilize or exploit them (those that are beneficial) or to protect ourselves from and control them (those that are pathogenic).

1- Phenotypic Classification

Since the classification proposed by Cohn in 1872 and until the early 1960s, all bacterial taxonomy was based on a phenetic classification. Phenetic (or phenotypic) classification groups organisms based on the similarity of their phenotypic characteristics. It uses a number of characteristics considered important :

- **Morphological aspect** : shape, size, presence or absence of a capsule, flagella, etc.
- **Structural aspect** : presence of mucopeptide, cell wall, etc.
- **Staining characteristics** : all types of staining (Gram, simple, etc.)
- **Trophic type** : aerobic, phototrophic, etc.
- **Metabolism** : carbohydrate, protein, etc.

Morphological characteristics are useful for identification but cannot alone demonstrate phylogenetic relationships.

1.1- Metabolic Tests :

Very important, they can distinguish closely related bacteria. The presence of enzymes (oxidase, catalase), the degradation of urea, esculin, the transformation of lactose and gas production, the use of different sugars as a carbon source, the use of citrate, and the production of acetoin are investigated.

These techniques have been miniaturized in specialized galleries (API), where up to 20 tests can be performed on a single gallery specific to enterobacteria **(Fig.1)**.

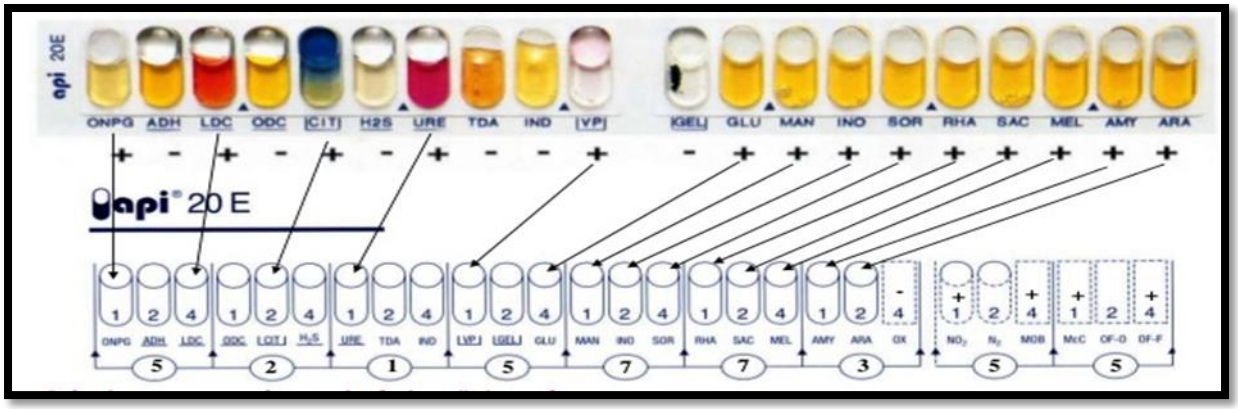

Figure 1 : Identification of bacteria using API biochemical gallery.

1.2- Serological Method :

Serodiagnosis and stereotyping are based on the specific antigen-antibody reaction. This method allows differentiation between species and even strains within the same species. The targeted antigens are O antigens in Gram-negative bacteria, H flagellar antigens, and K capsular antigens.

1.3- Inhibition Tests :

The growth of microorganisms is evaluated on selective media in the presence of antibiotics (antibiogram).

1.4- Chemotaxonomy

The fatty acid profile of the cell wall is determined. The total protein profile is analyzed by electrophoresis (separation based on pI and molecular weight).

1.5- Lysotyping

Infection by bacteriophages and formation of lysis plaques. The lysovar or lysotype is defined **(Fig.2)**.

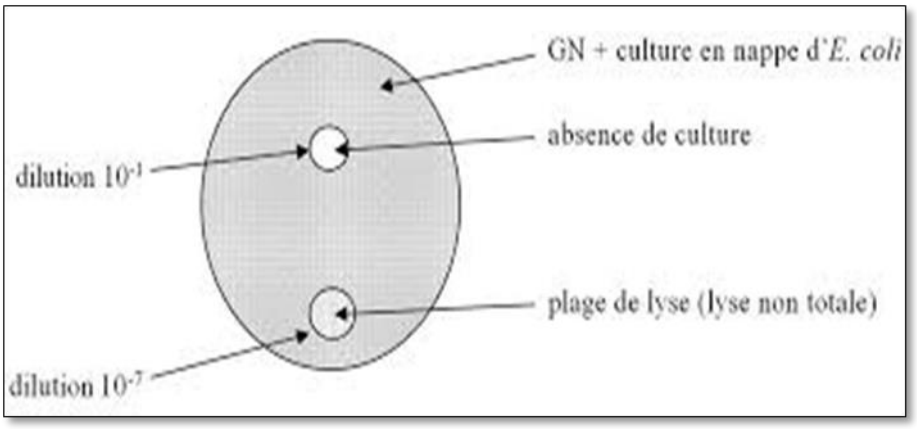

Figure 2 : Lysotyping of *E. coli* by a bacteriophage (absence of culture indicates lysis).

2. Genetic or Phylogenetic Classification

The genetic information of bacteria is carried by *nucleoid* and *plasmidic* genophores, which we refer to as the genome. In recent years, bacterial classification has been based on the structure of DNA, expressed by the *Chargaff coefficient*, which represents the percentage of Guanine and Cytosine in moles in the DNA molecule.

The criteria sought are :

1. Genome size.
2. DNA base composition in the form of G+C percentage (GC%).
3. DNA/DNA hybridization rate.
4. The sequence of DNA coding for 16S ribosomal RNA.

2.1. Genome Size

The genome size varies by species. For example, in paratrophic bacteria, the genome is very reduced.

2.2. DNA Base Composition (Chargaff Coefficient) :

Regardless of the species of origin, DNA always contains as much purine as pyrimidine :

(A + G) = (C + T) or (A+G) / (C+T) = 1

Moreover, there is as much thymine as adenine A/T = 1, and as much guanine as cytosine G/C = 1.

However, the ratio (A+T)/(C+G) varies greatly : it is characteristic of the species.

This coefficient is called the Chargaff coefficient. It can be calculated after sequencing using the formula ((G+C)/ (A+T+G+C)) X 100.

Or by ultraviolet spectrometry.

2.3. DNA/DNA Hybridization

Key temperatures and their definitions :

Tm : Melting point (Thermal elution midpoint) : Temperature at which 50% of the hybrid denatures.

Tor : Optimal renaturation temperature : 25° to 30°C < Denaturation temperature.

Trr : Restrictive renaturation temperature : 10 to 15 °C < Denaturation temperature **(Fig.3).**

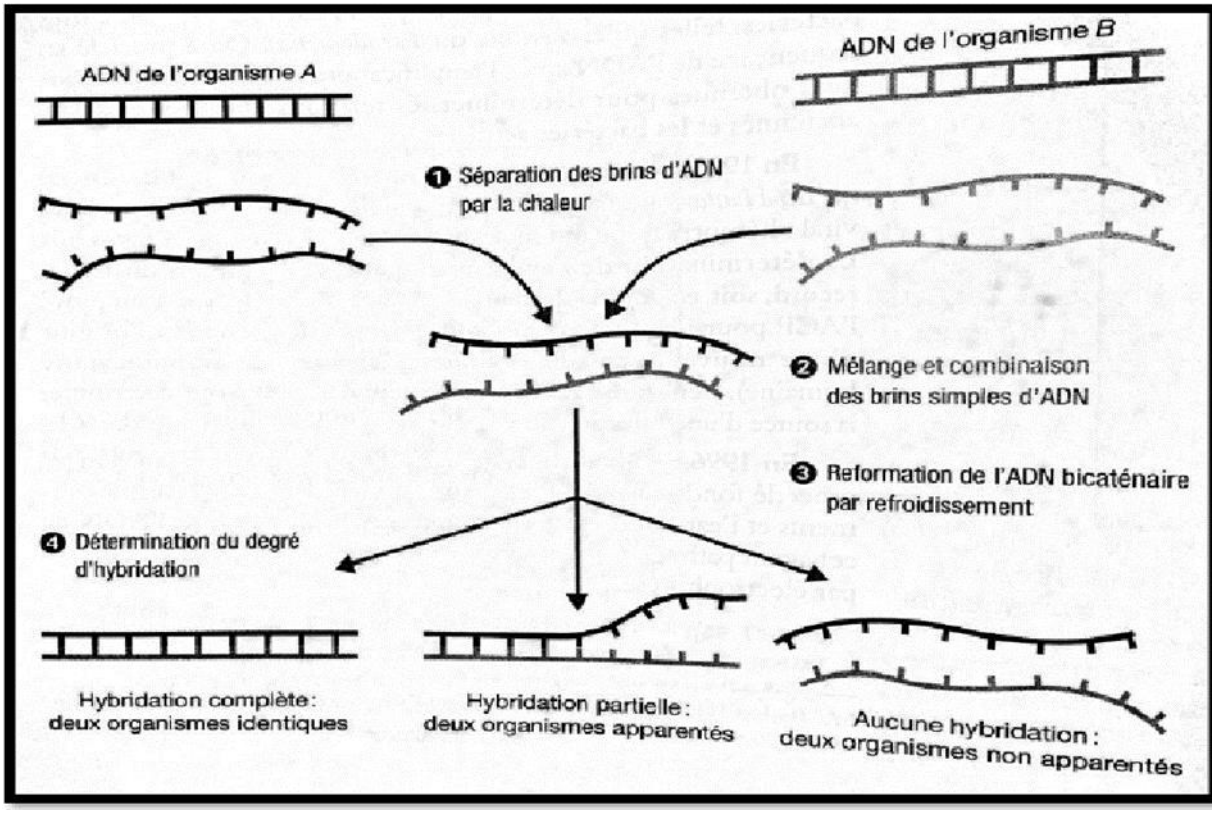

Figure 3 : Schematic explanation of the hybridization phenomenon.

3.4. Sequencing of Ribosomal RNA (rRNA)

According to Woese, rRNA is the best molecular clock due to :

The constancy of their function.

- Their distribution in all organisms.
- Their large size.

Taxonomists use a maximum of ecological, morphological, and metabolic characteristics, as well as molecular properties, to obtain the most reliable and realistic results.

4. Classification According to Bergey's Manual

Despite the challenges posed by bacterial classification, there are works in this field, the most notable of which is certainly the "Bergey's Manual of Systematic Bacteriology," proposed in 1923 in the USA, with the initial objective of grouping known bacterial species to facilitate the identification of unknown organisms. In its latest version (1984), the classification mode is phenetic and based on the determination of simple characteristics such as : Gram staining, reaction with oxygen, motility, sporulation, energy and nutrient sources. The latest classification by *Bergey* classifies bacteria in the class Schizomycetes, which is divided into ten orders, then into families, tribes, genera, and finally species (10 O.F.T.G.sp).

According to *Prévot*, bacteria are classified into four subphyla, each divided into classes, orders, families, tribes, genera, and finally species (4 sE.C.O.F.T.G.sp).

Krassilnikov divides bacteria into four classes, each of which is divided into families, genera, and species (4C.F.G.sp).